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# Phylogeny of the genus *Latrodectus* (Araneae: Theridiidae) and two new species from the dry forests in the Magdalena Valley-Colombia

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**ABSTRACT**

Black widow spiders (Theridiidae: *Latrodectus*) are known to wide audiences for their medical importance and to spider taxonomists for the striking variation in coloration and low differentiation in morphological structures like genitalia, setae number and location, and color pattern. Only two species have been described for Colombia: *Latrodectus curacaviensis* (Müller, 1776) and the widely distributed *L. geometricus* Koch, 1841, from the Caribbean coasts and mid-Andean valleys, respectively. The use of molecular data, for the first time on Colombian species, revealed two new species, *L. garbae* sp. nov. and *L. hurtadoi* sp. nov., which we further studied and describe here. A molecular phylogenetic analysis was conducted with Bayesian Inference using partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI), and the mitochondrial ribosomal RNA gene 16S (16SrRNA). Using the phylogenetically closest species, we checked for reciprocal monophyly based in clade positions, estimated genetic divergence, Fst, and identified fixed sites within each gene. All species were genetically compared with the closest phylogenetic relative to give enough taxonomical validation to the new species hypothesis. Despite the limited value of morphological traits in *Latrodectus* taxonomy and species delimitation, we provide here a detailed morphological description of male and female sexual organs, as well as general body traits for the two new species. New reports are made for *L. geometricus* and *L. curacaviensis* in 10 departments in the country.

**Key words:** Molecular phylogeny, Morphological description, Black widow.

**Short summary:**

The first approach to black widow systematics knowledge in Colombia. Two new species of *Latrodectus* are described using morphological and molecular

information, and new localities are reported. Genetic distances are calculated between related taxa. All our results support the status of new species of *L. garbae*, **sp. nov.** and *L. hurtadoi*, **sp. nov.**

## 1. INTRODUCTION

Black-widow spiders of the genus, *Latrodectus* Walckenaer, 1805 (Araneae: Theridiidae), is widely known due to the powerful venom they produce. *Latrodectus* contains 32 species described to date (World Spider Catalog 2020) including the recently described *Latrodectus umbukwane* from South Africa (Wright *et al.* 2019). The taxonomy of the genus remains complex, because much of the variation in coloration, abdomen color patterns, and the abundance of setae in the abdomen, appears to be continuous throughout wide geographical ranges (Levi 1959). Very early, 22 extant species had been synonymized into just six by Levi, many of which were considered cosmopolitans (Levi 1959). Although taxonomists initially paid particular attention to the female and male genitalia (epigynum and pedipalps), the high similarity in reproductive structures for most species undermined their value for species identification (Levi 1983). Molecular techniques have thus become a crucial tool for the determination of species boundaries and recognition of new species in the genus (Garb *et al.* 2004).

Two species of *Latrodectus* are currently listed in Colombia, *L. curacaviensis* (Müller, 1776) and *L. geometricus* Koch, 1841. There are also reports of Latrodectism syndrome, but the species involved are not reported (Aguirre-Plata 1914; Hamburger 1938; Quintana & Otero 2002; Marie & Vetter 2015). *Latrodectus* spiders have been reported in dry forests of Colombia (Quintana & Otero 2002; Rueda *et al.* 2017) including the arid environments in Tatacoa (Aguirre-Plata 1914; Hamburger 1938; Gilij 1955; Boussingault 1985). During field expeditions in Colombia, we found populations of *Latrodectus* with a distinctive color pattern not resembling any of the species present in Colombia. Since coloration and reproductive structures are not sufficient traits for species description, we conducted further molecular analysis on collected specimens that erected them as new species. The new morphotypes were found in dry to very dry forests: Tatacoa desert (Huila) and Mesa de los Santos (Santander). At about 440 meters above sea level (masl), Tatacoa desert is characterized by two rainy seasons per year with annual rainfall between 1000–1500 mm (average data from 1981–2010, IDEAM 2017). The *Latrodectus* spider found here had been reported in medical articles more than a century ago, under the common name of “coya” (Aguirre-Plata 1914; Hamburger 1938). Its venom was described as strong and able to produce mortal spasms (Gilij 1955). The small rate of human accidents with this dangerous species is intriguing, even casting doubts on its existence (Boussingault 1985). At about 1480 masl, Mesa de los Santos receives less than 1000 mm of annual rainfall (IDEAM 2017); the intense solar radiation, strong winds, and high evapotranspiration favors arid conditions (Cuatrecasas 1958; Casas-Pinilla & Ríos-Málaver 2017). Also, a population of *L. curacaviensis* was found in Mesa de los Santos in sympatry with *L. geometricus*. There are no previous reports of any accident related with venomous spiders, or any historical records of the colonization or establishment of a population of black widow spiders in this region.

Dry forests are considered one of the more endangered, degraded, fragmented, and less studied ecosystems in Colombia; and they have less than 8% of the original habitat remaining, according to the Biological Resources Research Institute Alexander von Humboldt (Pizano *et al.* 2014). There are three big regions with dry forests in the Country: the Caribbean Plain with most of the coverage, the Magdalena Valley with special focus in Tolima, Cundinamarca and Huila, and the Cauca Valley where only little fragmented remnants can be found. Most of these lands are used for agriculture and cattle raising (IAVH 1998). More studies are needed about the vegetation, fauna and flora that belongs to these regions to develop conservation programs and restoration plans.

In addition, little is known about the natural history, ecology, and biology of *Latrodectus* in the country. The lack of sound information on the genus, as well as its ecological and medical importance, offer enough justification to test the taxonomic identity of these morphotypes and to formally describe them. We describe two new *Latrodectus* species collected in dry forests in Colombia, and further provide a phylogenetic framework based on mitochondrial cytochrome oxidase I (COI) and ribosomal 16S rRNA for our discussion, including new reports for currently recognized species and the new taxa. Also, sequences belonging to *L. curacaviensis* are available for the first time for a molecular study. This study aims to increase the knowledge of this genus in Colombia and understand the genetic relation between all populations and species related with the identified taxa that were sampled.

## 2. MATERIALS AND METHODS

We conducted field work to sample the major dry forests of the country that were comparable with regard to climatic conditions (Pizano *et al.* 2014, see Table S1) to the previously known localities of *Latrodectus*. With the information gathered about the location of the remnants of dry forests, the study centered the sampling sites to the Magdalena Valley and the Caribbean Plains, including

some sampling sites where isolated populations were found in the Eastern Plains and the Pacific foothills of the Andes Mountain Chain (Fig. S4).

Most webs were found within 50 cm above the ground and anchored on rocks or small plants (Rueda *et al.* 2017). Only *L. geometricus* was found near or within human habitats. Between 2010–2016, we collected specimens in absolute ethanol. All samples were preserved at -20°C in the Zoology and Aquatic Entomology Lab (LAZOEA) at the Universidad de los Andes (Bogota, Colombia). Preserved specimens were later deposited at the Natural History Museum ANDES, in the invertebrate collection (Universidad de los Andes).

#### DNA extraction, PCR amplification and sequencing:

The legs or cephalothorax of each specimen were used for DNA extraction with the Qiagen DNeasy Blood & Tissue Kit according to manufacturer's protocol. (Valencia, CA, USA). The universal primers for the mitochondrial protein-encoding gene cytochrome c oxidase subunit I (COI) (Folmer *et al.* 1994): LCO1490: 5' GGT CAA CAA ATC ATA AAG ATA TTG G 3' and HCO12198: 5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3', and for 16SrRNA (Xiong & Kocher 1991): 16Sar: 5' CGC CTG TTT AAC AAA AAC AT 3' and 16Sb: 5' CCG GTT TGA ACT CAG ATC ACG T 3' were used at 10 µM for the amplification of the mitochondrial and ribosomal genes. The PCR conditions used in an INFINIGEN thermal cycler were as follows: initial denaturation at 94°C during 3 min, 35 cycles of 94°C during 45 s, 50°C during 50 s, and an extension of 72°C for 1 min, ending with a final extension of 72°C during 5 min for COI. Annealing temperature for 16S was of 47°C keeping the rest of the cycle as for COI. The PCR products were purified using the EXO-SAP method and sent for sequencing to MACROGEN INC. (Seoul, Korea). The chromatogram assessment and contig definition were performed using Geneious R 11 (Kearse *et al.* 2012). DNA sequences were uploaded to GenBank (see Table S1 for accession codes). For several *Latrodectus* species, we further received DNA aliquots from Jessica Garb (Garb *et al.* 2004) and whole specimens (*L. hesperus* Chamberlin & Ivie, 1935) from EVOLAB, University of California, Berkeley, USA.

#### Phylogenetic reconstruction and genetic analysis:

*Latrodectus* COI sequences were downloaded from NCBI and combined with our own sequences (Table S1). The outgroup taxa selected were *Crustulina sticta*, *Steatoda bipunctata*, *Steatoda borealis*, *Steatoda grossa*, and *Theridion longipedatum*. The selection was made based in the previous phylogenetic analysis made by Garb *et al.* 2004, and in order to include taxa with sequences available for 16SrRNA two taxons were selected from Arnedo *et al.* 2004; both belonging to sister groups: *Crustulina sticta* and *Theridion longipedatum* that was also a specimen collected in Colombia.

The whole set (COI and 16SrRNA) was cleaned and assembled using Geneious R 11 (Kearse *et al.* 2012) and aligned using Clustal W (Thompson *et al.* 1994), creating three matrices, one for COI, one for 16SrRNA and one with a concatenated alignment. All new sequences were made available on GenBank (sequence codes in Table S1).

A test for model evolution was performed for each matrix with JModelTest (Posada 2008); we used the corrected Akaike Information Criterion, AICc to select the substitution model that better fitted the samples. A phylogenetic analysis with Bayesian Inference was conducted on BEAST 1.8.4 (Drummond & Rambaut 2007), using 50 million generations and sampling trees each 1000 generations; the priors used for the Bayesian analysis included Tree prior: Coalescent: Constant size with a random starting tree and the information of the model obtained for each set of sequences. Burn-in was calculated using TRACER (Rambaut *et al.* 2014). The criteria used to assess stationarity in TRACER, was analyzing the trace file and establishing a ESS lower limit in 150 for the posterior probability.

Kimura 2-Parameters genetic distances were calculated among studied species with Mega 7 (Kumar *et al.* 2015). A threshold of 2% of genetic difference was used as molecular evidence to separate lineages (Barrett & Hebert 2005). Median Joining Networks for COI and 16SrRNA sequences were built using PoPArt software (Leigh & Bryant 2015). Also, genetic indexes such as Fst and Haplotypic diversity were calculated using DNAsp 5 (Librado & Rozas 2009).

#### Morphological methods:

The female epigyna were dissected and cleared using a solution of 10% KOH, leaving them in simmer for five days with periodical checking on the pieces, and then clearing in clove oil. Male palps were expanded through repeated immersions in the KOH solution and distilled water (Cooke, 1970; Levi 1965). We then noted character description for male pedipalp structure, form and measurements, external and internal female genitalia structure, form and measurements, and color pattern of the abdomen, legs and cephalothorax; the structures and general morphology were photographed and measured using a Leica EZ4HD stereoscope and camera lucida for the illustrations of the genitalia. Measurements were taken in millimeters (mm) for 20 female individuals per species for *L. garbae* sp. nov., *L. hurtadoi* sp. nov., *L. curacaviensis*, 10 males for *L. garbae* sp. nov., 4 males for *L. hurtadoi* sp. nov., and 5

males for *L. hesperus*. Measurements include carapace length and width, cephalothoracic coefficient, patella length, tibia length, sternum length and width. Body size was inferred from the carapace width (Hagstrum 1971). Reference measurements from sister species for comparison were taken from available information in published articles and original description papers. No statistical test was performed to analyze the morphological variation between sister species because not enough replicas were found in published articles. Leg I measurements were made for the *curacaviensis* clade including the species *L. garbae* sp. nov. (N=20), and *L. curacaviensis* (N=20). Measurements for the species *L. diaguita* (N=1), *L. corallinus* (N=2) were obtained from Abalos (1980).

#### Abbreviations:

*Carapace or cephalothorax length*: Length of the carapace following the longitudinal axis, not including the chelicerae.

*Carapace or cephalothorax width*: Width of the carapace in the point of greater amplitude.

*Cephalothoracic coefficient (T)*: cephalothorax length not including chelicerae, divided by width at the point of greater amplitude (Melic 2000).

*Coefficient TT*: length of the tibia of leg I plus the length of the patella of leg I, divided by the length of the carapace following the longitudinal axis, not including the chelicerae (Melic 2000).

*Sternum length*: Length of the sternum following the longitudinal axis.

*Sternum width*: Width of the sternum in the point of greater amplitude.

*Patella*: length of the patella of leg I.

*Tibia*: length of the tibia of leg I.

*Metatarsus*: length of the metatarsus of leg I.

*Tarsus*: length of the tarsus of leg I.

*Femur*: length of the femur of leg I.

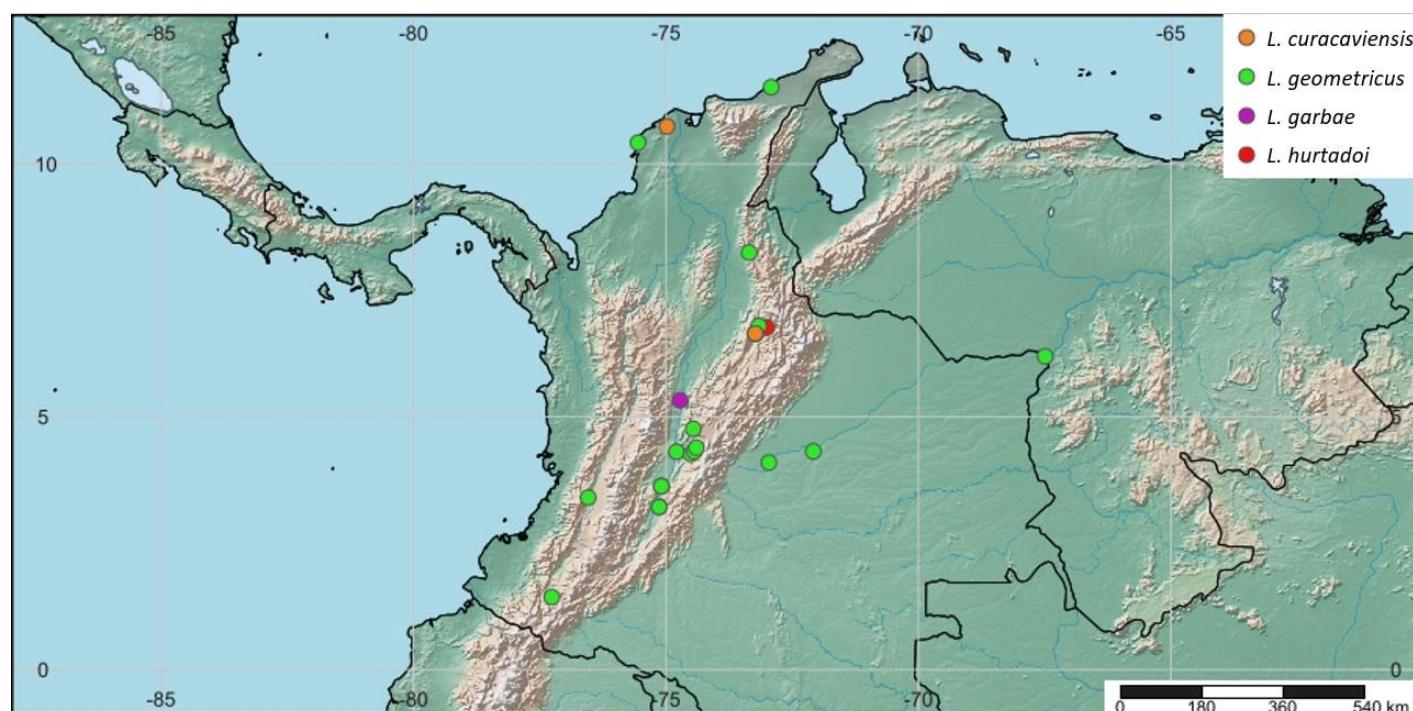
*Cymbium width*: Width of the cymbium in the middle point.

*Cymbium Length*: Length of the cymbium from the patella insertion to the furthermost point.

*Cymbium biggest width*: Width of the cymbium in the point of greater amplitude.

### 3. RESULTS

Spiders were found in dry to very dry forests across the Magdalena Valley and the Caribbean Plain. Also, isolated populations were found in the Eastern plains (Fig. 1.). All webs were small and were found near the ground for all species except *L. geometricus* that showed webs that reached a size of 2 meters in height and height adaptation to modified places and human settlements.



**Figure 1.** Location of the type locality of the two new species of *Latrodectus* sp.; *L. hurtadoi* sp. nov. at Mesa de los Santos in the northwest (red dot); *L. garbae* sp. nov. at the Tatacoa desert in the southeast, and two more populations (purple dots). New reports for *L. geometricus* (green dots) and *L. curacaviensis* (orange dots).

Sympatry was common phenomena inside of *Latrodectus*, most populations showed the presence of more than one species. Webs were not near one to another, and differences in height, size and location were noted. In Mesa de los Santos, three species were found co-existing: *L. geometricus*, *L. curacaviensis* and *L. hurtadoi* sp. nov., each found in different places, near human constructions, under rocks near to the ground, and cliffs respectively.

In the Tatacoa desert, *L. garbae* sp. nov. and *L. geometricus* were found living in sympatry until 2016. It was common to find a female spider of one species as a prey in the web of a female belonging to the other species.

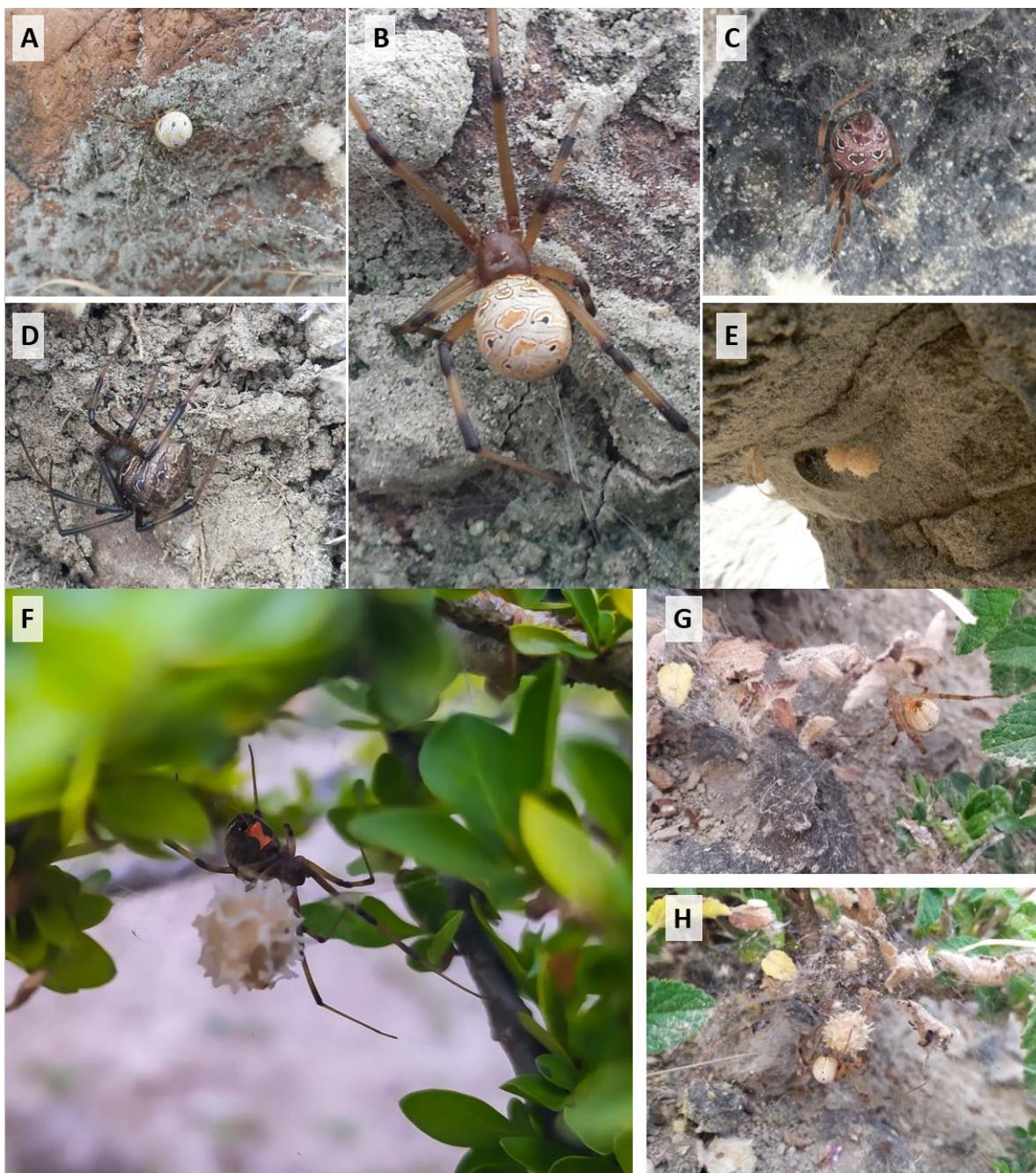
### Morphological analysis

*L. geometricus* shows a color polymorphism in the abdominal portion of the body, even inside the same population. The abdominal colors found show a range from very light green, yellow, orange, reddish and dark brown to black almost shadowing the lined pattern in the sides and the oval light spots found dorsally (Fig. 2.). Also, the colors inside of the spots in the dorsal abdominal pattern are related to the color of the abdominal background, for example if the background is reddish the spots will show a dark red color surrounded by light pink lines (Figs. 2D, 2F.). The hourglass in the ventral portion of the abdomen, did not show a color change between all the polymorphisms being light red and well defined in all specimens caught (Figs. 2F, 2G). The legs color is also homogeneous in all polymorphisms being light to dark brown with darker markings in the leg's articulations. The cephalothorax is also homogeneous in color in the ventral and dorsal portion of the body, showing a light to dark brown color with no change in the dorsal furrow. *L. geometricus* was the only species with a spiked egg sac and a nest formed of dense web with one entrance (Fig. 2E.). This species was found in conserved spots of dry forest between rocks and in plants between the leaves, also, it was the only species found near human constructions, and was the species that showed the biggest sized web with threads found in a door from the top to the ground with a length of 2 meters. The egg sac shows the characteristic spiked surface (Fig. 2G).

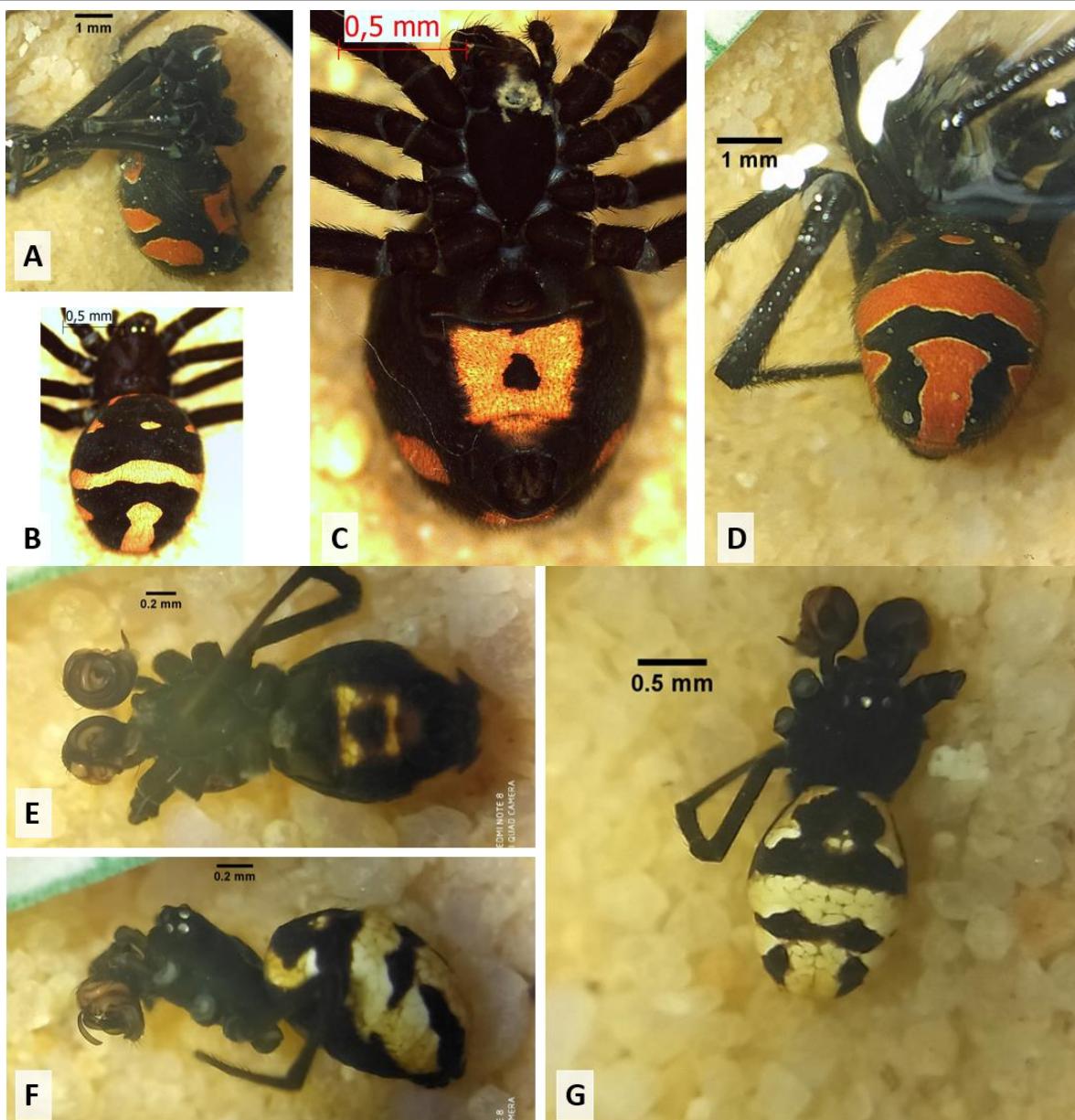
*L. curacaviensis* was found near the ground in dark places under rocks or small bushes in small sized webs with a maximum height measured of 35 cm above the ground. Color pattern

The color pattern is congruent with that described by Bhatnagar & Rempel (1962); the cephalothorax is dark brown to shiny black, the legs of the same color with no change in tone at the joints (Fig. 3). The abdomen shows a deep red background with black lines on the dorsal portion. There are two parallel black lines in the middle abdominal portion in the longitudinal axis, the posterior line joins with two small lines and shows a figure similar to the letter of the Greek alphabet  $\pi$  pointing towards the spinnerets (Figs. 3B, 3D). Near the pedicel, three red fractions are formed, the central one with an oval shape and the two lateral ones with triangular to rounded shapes, followed by two red spots that reach the pedicel, the latter may be attached to the lateral spots (Figs. 3A and 3D). These red spots are surrounded by a clear yellow line in the first stages of life that is lost when the spider reach maturity. All of the black lines meet in the mid-lateral abdomen to give a shiny black background to the ventral portion of the body (Fig. 3C). The hourglass has a trapezoid shape that surrounds a single black point in the center, at its rear limit there is a small extension of the same red color that approaches the spinettes (Fig. 3C). The coloration of the males follows the same coloration pattern that the females show (Fig. 3G).

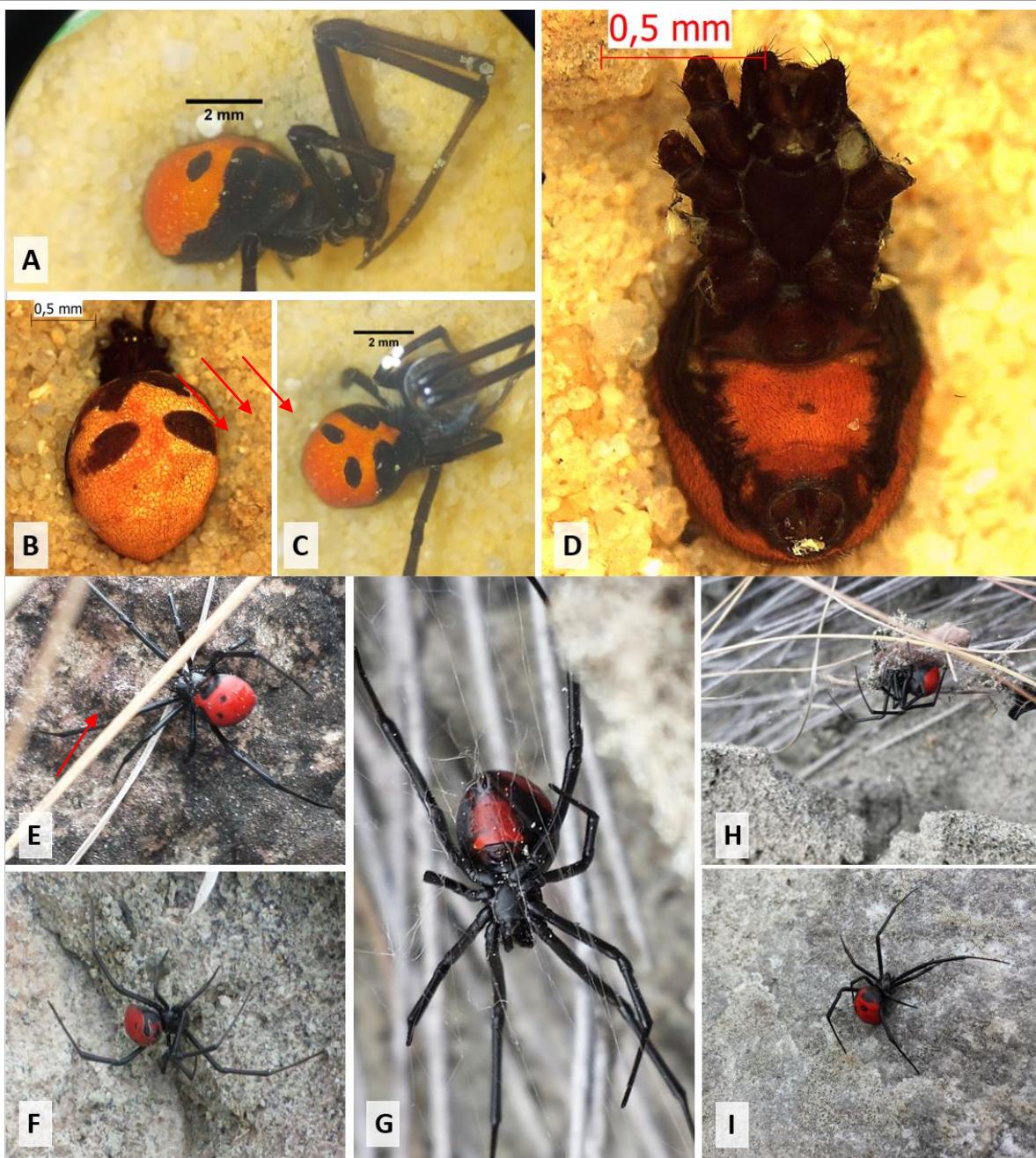
*L. garbae* sp. nov. has a dark brown to shiny black cephalothorax with no color or hue change in the legs or joints (Fig. 4). The dorsal portion of the abdomen has an intense red background with two rows of three oval to circular black spots parallel to the longitudinal axis of the body, the two rows are parallel and are located in the anteroposterior axis of the abdomen (Fig. 4C). These spots are surrounded by a light yellow halo in the juvenile stages, which is lost when the last molt is reached (Fig. 4F). As maturity progresses, the larger spots (medial close to the pedicel) can merge with the black background of the ventral abdomen while still having the yellow halos around them (Fig. 4F). In the last molt, the anterior and medial spots of each row can merge with the black background of the ventral portion of the abdomen, forming an arrow pointing to the pedicel (Fig. 4E). The posterior spots are the smallest, coming to appear in the form of points and in some specimens, they are absent (Fig. 4B). The ventral portion of the abdomen shows a black background with a rounded hourglass, the antero-posterior edges reach from the spinettes to the base of the epigynium in females, in males a defined clock with marked edges can be observed (Fig. 4D). The coloration of the males is similar to that described for the females, the cephalothorax and the legs are dark brown to glossy black, with no variations in hue or color in the joints (Fig. 11E). The pattern of spots on the abdomen keeps the two rows of round spots parallel to the longitudinal axis of the body that do not merge at maturity. The pedipalps are round and large in color, dark brown or glossy black (Figs. 11F and 11G).



**Figure 2.** *L. geometricus* field pictures of different polymorphisms found in the abdominal background: A. Light yellow to white; B. Bright yellow; C. Red; D. Dark brown to black; E. Nest and spiked egg sacs; F. Dark colored spider showing the bright red coloration of the hourglass and the spiked egg sac in the web surrounded by leaves; G. Nest with dry leaves and organic matter, female showing the bright red hourglass; H. Nest with organic matter and a female with a spiked egg sac. Field pictures, no scale bar available.



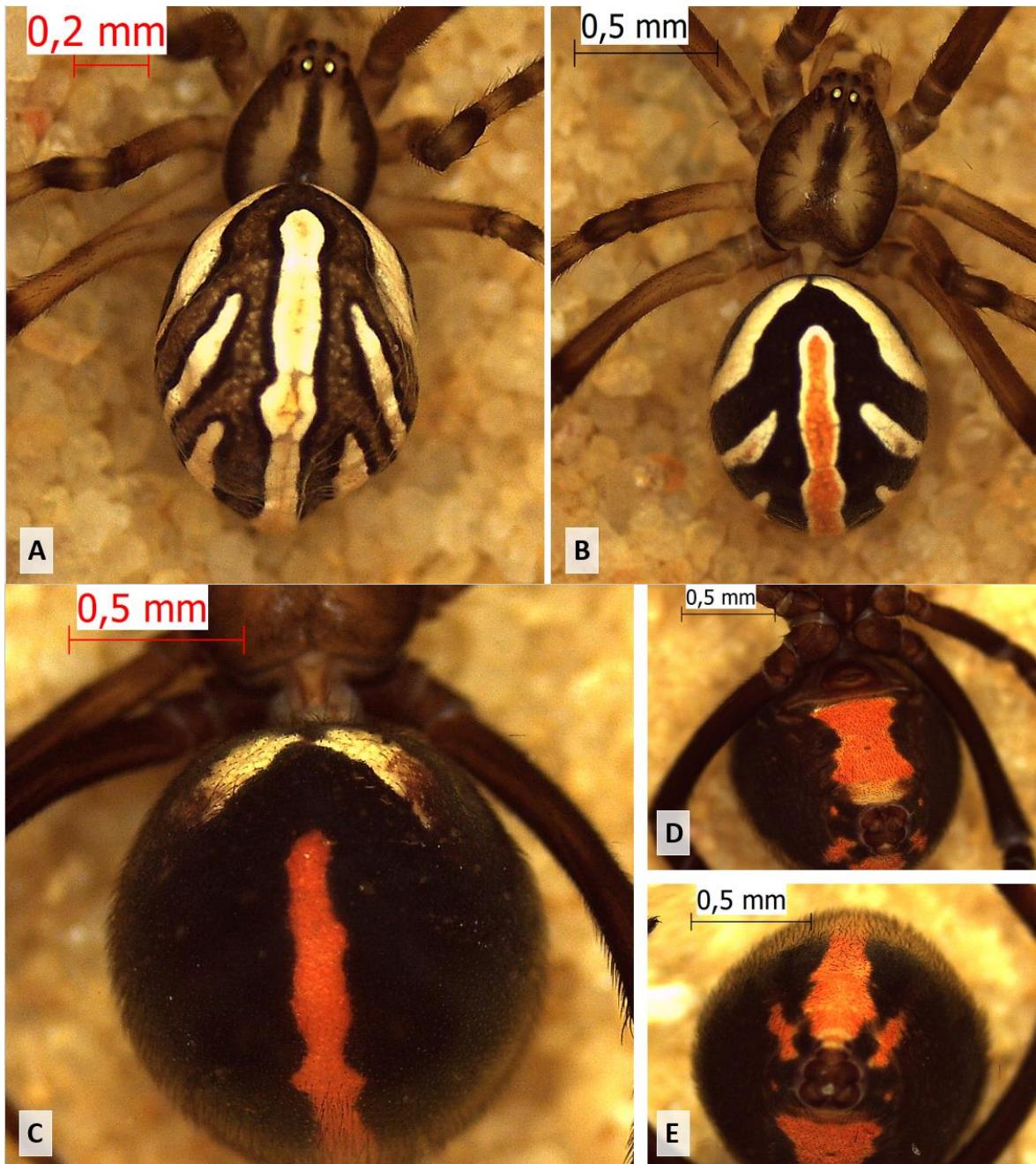
**Figure 3.** *L. curacaviensis*: A. Female, lateral body view; B. Mature female, dorsal body view; C. Mature female, ventral view; D. Juvenile female, dorsal view; E. Male, ventral view; F. Male, lateral view; G. Male, dorsal view.



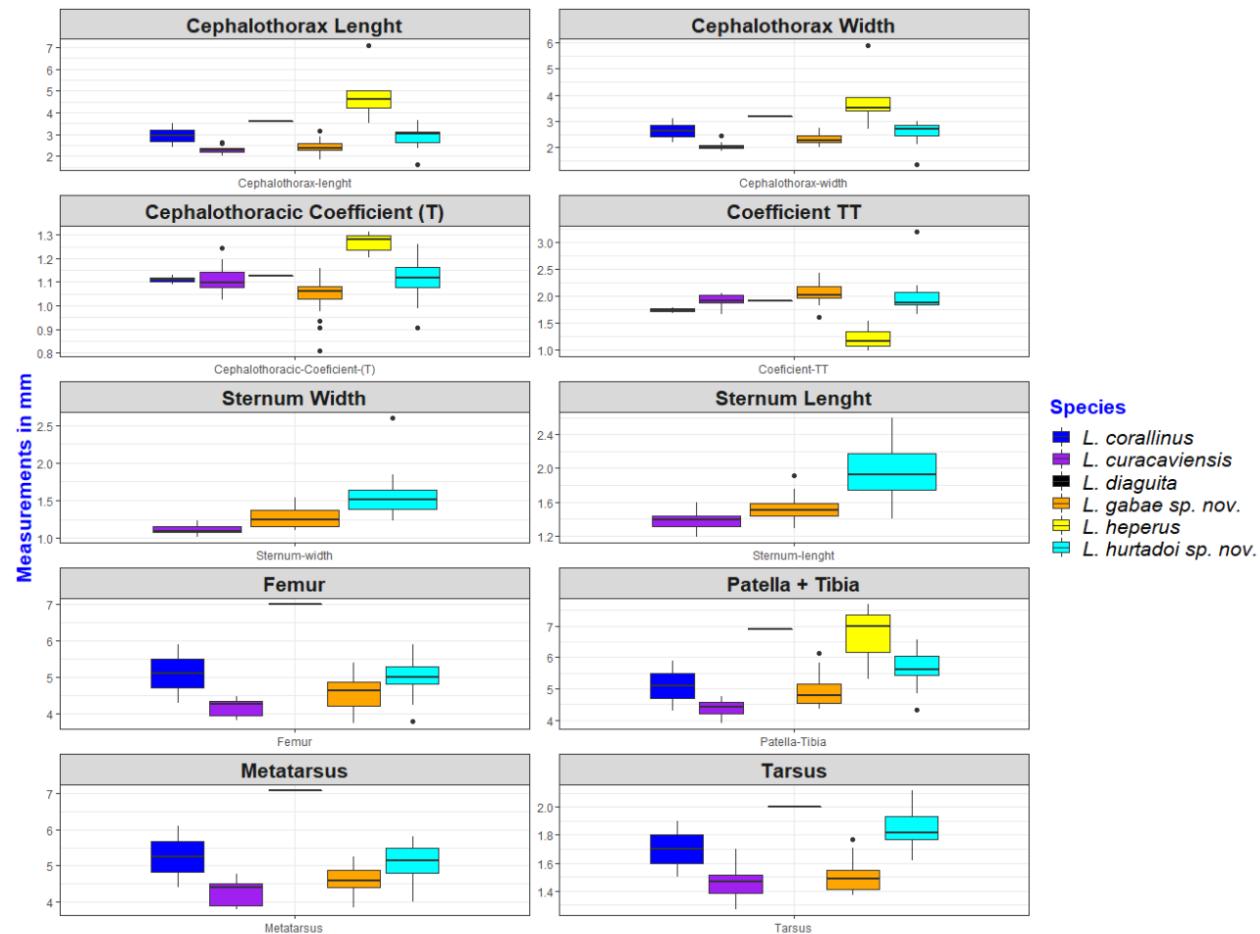
**Figura 4.** *L. garbae* sp. nov.; A: Adult female lateral view; B: Adult female dorsal view, last 2 spots very small; C: Adult female dorsal view, arrows pointing at the 6 black spots on the dorsal coloration pattern; D: Adult female ventral view; E: Adult female dorsal view, arrow like color pattern due to merging of the spots during ontogeny; F: Juvenile female lateral view, yellow halos around the black sports; G: Adult female ventral view; H: Adult female furrow with organic material; I: Adult female lateral view.

*L. hurtadoi* sp. nov. presents a color pattern that changes with ontogeny in a similar way to what is presented *L. Hesperus* (Fig. 5). Spiderlings show a light brownish coloration, with three lateral orange, red or white stripes surrounded by a darker border that is present surrounding the ventral hourglass shape (Figs. 5A and 11A). These stripes follow the transversal plane of the body. A fourth stripe is present in the longitudinal axis of the abdomen, from the pedicel to the spinnerets. The caparace is light brown with darker portions around the carapace furrow and sternum. The coloration changes after some molts the background starts to turn dark black and the stripe coloration fades to white; the longitudinal strip and the one nearer to the pedicel show a red color appearing in the middle (Figs. 5B and 11B). Cephalothorax colorations remain the same as the spiderlings during these molts. When maturity is reached the only stipes remaining are the ones near the pedicel with a white coloration and the longitudinal stripe with a red coloration (Fig. 5C). The hourglass changes also to a deep red color with no border (Fig. 5D). Near the pedicel, two small red stripes and points remain (Fig. 5C). Cephalothorax color turns darker and homogeneous.

The number of measurements made of mature female spiders are: *L. corallinus*: 2 (Abalos, 1980), *L. diaguita*: 1 (Abalos, 1980), *L. curacaviensis*: 10, *L. garbae* sp. nov.: 20, *L. hesperus*: 10, *L. hurtadoi* sp. nov.: 20 (Fig. 6.); and for mature male spiders are: *L. corallinus*: 1 (Abalos, 1980), *L. diaguita*: 1 (Abalos, 1980), *L. garbae* sp. nov.: 5, *L. hesperus*: 5, *L. hurtadoi* sp. nov.: 4. Measurement analysis shows that the species *L. hurtadoi* sp. nov. shows the highest values for all available data in female and male measurements (Figs. 6 and 7). *L. hurtadoi* sp. nov. shows more similarities in size with *L. corallinus* for the female measurements than with the close relative *L. hesperus*. The smaller females measured belong to *L. curacaviensis*, followed by *L. garbae* sp. nov. *L. diaguita* only has measurements for the type specimen that seems to be a very large spider following the behavior of a statistical outgroup, more measurements are required in order to have a certain idea of the real size of these spiders. General morphology tends to stay homogeneous with no variations between the width and length of the females.



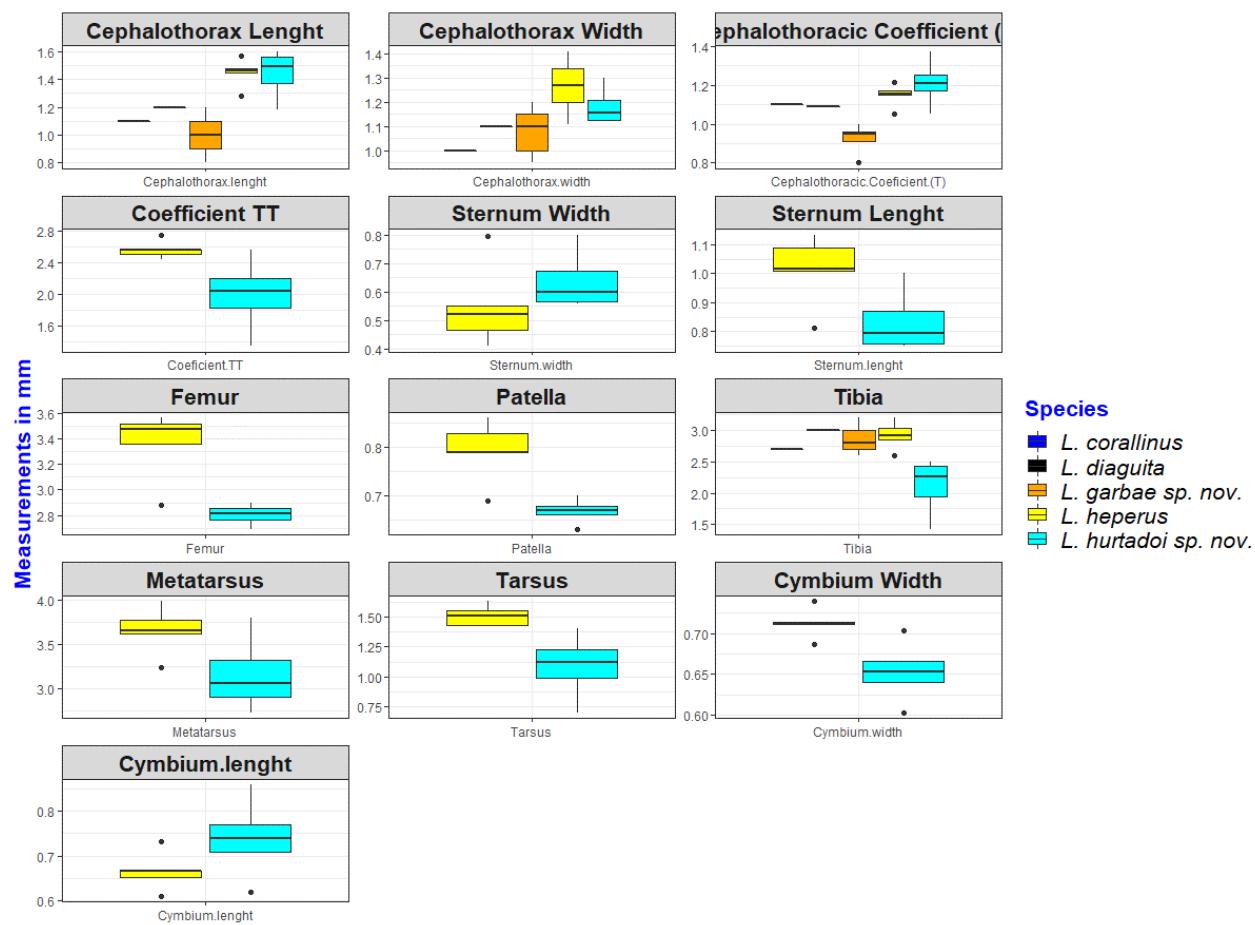
**Figure 5.** *L. hurtadoi* sp. nov.; A: Female spiderling; B: Female juvenile; C: Adult female dorsal view; D: Adult female ventral view; E: Adult female spinnerets.



**Figure 6.** Female measurements. *L. corallinus* (N=2)\*, *L. diaguita* (N=1)\*, *L. curacaviensis* (N=10), *L. garbae* sp. nov. (N=20), *L. hesperus* (N=10), *L. hurtadoi* sp. nov. (N=20).

\* Information extracted from Abalos, 1980.

Between males (Fig. 7.), the sternum width is larger for *L. hurtadoi* sp. nov. than it is for *L. hesperus*, while the sternum length is bigger for *L. hesperus* than for *L. hurtadoi* sp. nov.; the male spiders of *L. hesperus* wider but the male spiders of *L. hurtadoi* sp. nov. are longer and thinner. For the leg measurements, the relation shows that *L. hesperus* legs are longer in all their sections than the legs of *L. hurtadoi* sp. nov. Also, the cymbium of *L. hesperus* is wider and shorter with a rounded symmetry; the cymbium of *L. hurtadoi* sp. nov. is longer than wider, forming an oval. *L. corallinus* shows the smallest cephalothorax width measurement and keeps a very short distance from the cephalothorax coefficient (T) with *L. diaguita*. *L. garbae* follows in size, with the smallest value measured for cephalothorax coefficient (T). The measurements show that the cephalothorax of *L. garbae* is wider and shorter unlike the other *Latrodectus* species. The morphological analysis is described in detail in the taxonomy description of the two new species at the end of the document. For average measurement information please see Tables S7-S10.



**Figure 7.** Male measurements. *L. corallinus* (N=1)\*, *L. diaguita* (N=1)\*, *L. garbae* sp. nov. (N=5), *L. hesperus* (N=5), *L. hurtadoi* sp. nov. (N=4).

\* Information extracted from Abalos, 1980

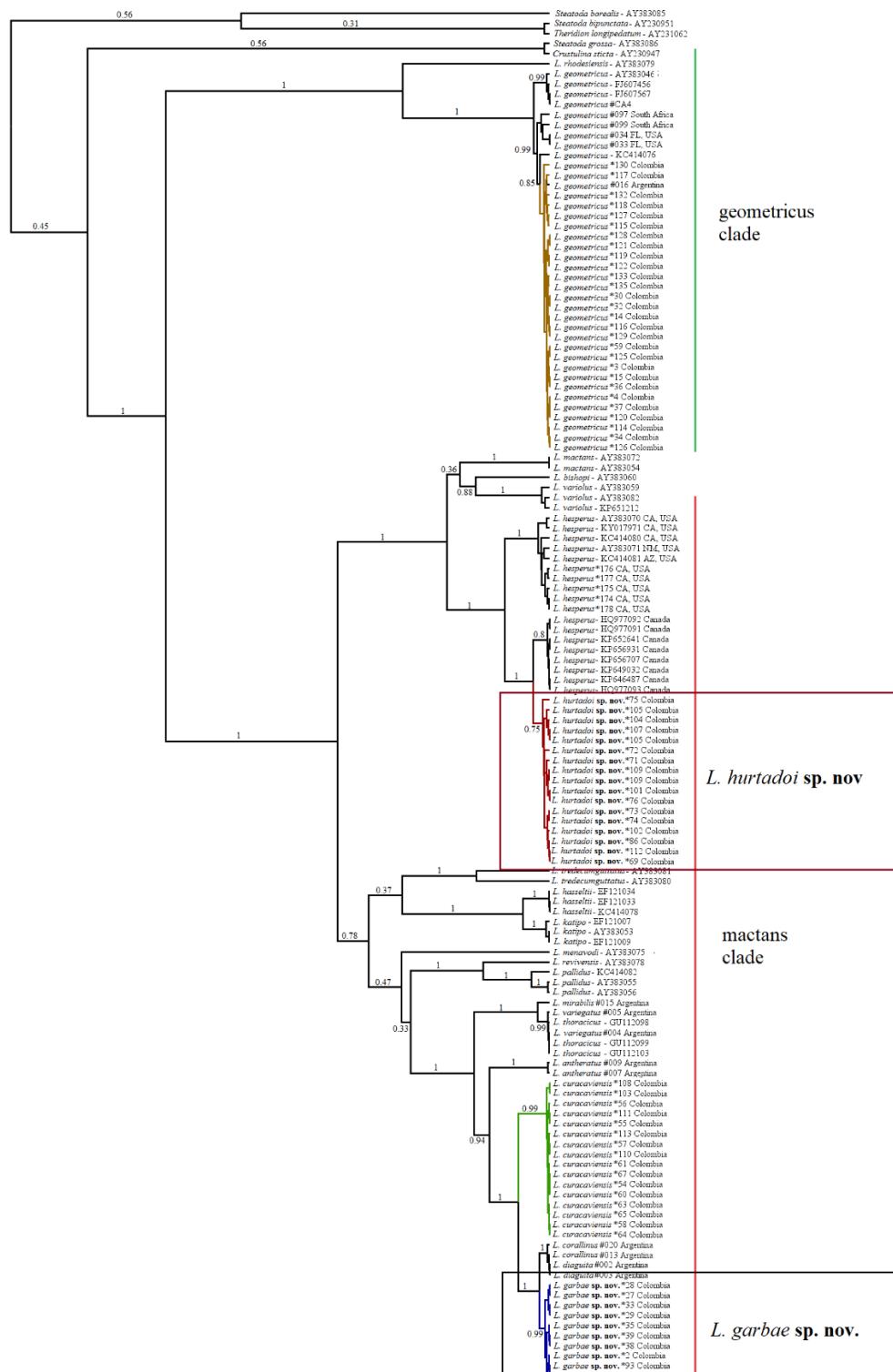
#### Intrageneric Phylogenetic relationships

We found evidence to recognize four species of *Latrodectus* in Colombia, two of them already described: *L. curacaviensis* and *L. geometricus*. They were found along the Magdalena Valley mostly in dry and warm places. Sympathy is a common phenomenon in *Latrodectus* species across the country. All species show differences in ecosystem preference and appearance near human constructions (Fig. 1). The only species occurring in habitats with a wide range of temperatures was *L. geometricus*, from Silvania, Cundinamarca (average temperature of 19°C) (IDEAM 2017) to Tatacoa (average temperature of 37°C) (IDEAM 2017). We found new populations for *L. geometricus* in 10 localities, and for *L. curacaviensis* 2 new localities; Bucaramanga and Mesa de los Santos, Santander; previous reports show populations in Usiacurí, Atlántico (Escoria-Gamarra and Matinez 2013). (see Table S1 and Fig. 1). The new species found in the Tatacoa desert, *L. garbae* sp. nov. was first found living in sympatry with *L. geometricus* in the Tatacoa desert, but after 2016 no specimens of *L. garbae* sp. nov. were found again in that site.

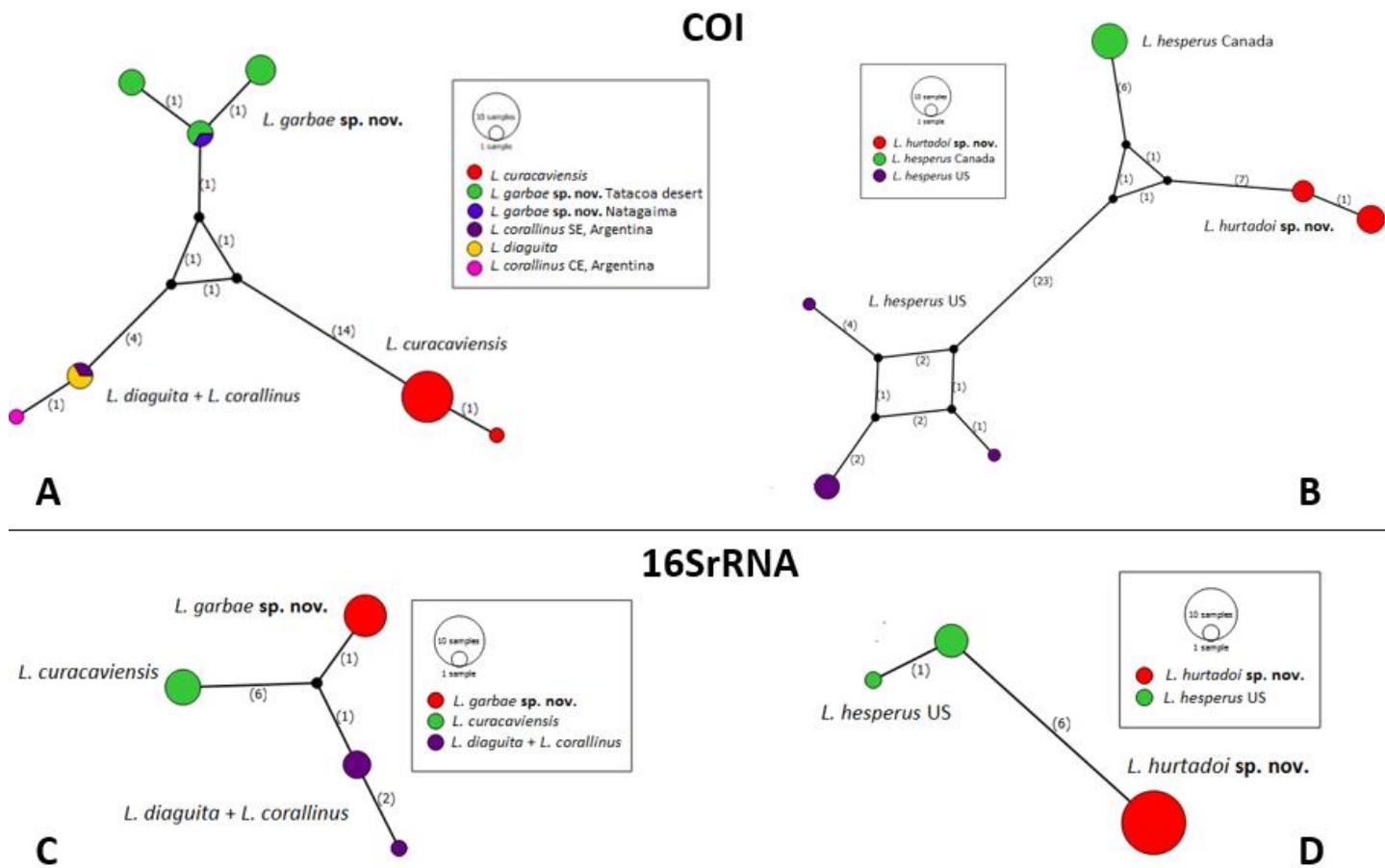
**Species delimitation:** A total of 112 COI (650bp length for this study sequences/ 400bp length for sequences from GeneBank) and 69 16S rRNA (400bp length) sequences were used for the phylogenetic inference analyses. All new sequences are available on GenBank (Table S1). The best substitution model according to AICc test for COI was TMV+I+G; for 16S was GTR+G. The phylogenetic hypothesis resulting from the concatenated alignment (Fig. 8) grouped together all *Latrodectus* species with a posterior probability (PP) of 1. The two major clades described in Garb *et al.* (2004) were recovered with a PP of 1: geometricus and mactans.

In addition to the currently named species, we recovered two monophyletic groups of Colombian samples. One group belonging to *L. garbae* sp. nov. (MT1) occurred in and near Tatacoa (Departments of Huila and Tolima, central Andes of Colombia) in sympatry with *L. geometricus*; sister to a clade composed of the Argentinian species *L. corallinus* Abalos, 1980 and *L. diaguita* Carcavallo, 1960. Also, *L. garbae* sp. nov. was in turn reciprocally monophyletic to *L. curacaviensis* for all trees (Figs. 8, S1 and S2). These three groups (*L. garbae* sp. nov., *L. curacaviensis* and *L. diaguita*+*L. corallinus*) form what we will refer to as the curacaviensis clade with a PP of 1 for all topologies. The curacaviensis clade is inside the mactans clade defined by Garb *et al.* 2004. *L. antheratus*

(Badcock, 1932) is sister group to the *curacaviensis* clade with a PP of 0.94 in the concatenated alignment (Fig. 8) and with a pp of 0.94 in the COI analysis (Fig. S1). For the 16SrRNA gene fragment this species groups *L. mirabilis* (Holmberg, 1876) + *L. variegatus* Nicolet (1849) (PP= 0.64) (Fig. S2). To complete the South American clade, we recovered a monophyletic group (PP=1) with non-resolved relations in the inside, where *L. mirabilis* is sister to a non-resolved clade that contains *L. thoracicus* and *L. variegatus*, more studies are needed to resolve and evaluate possible synonymy (Fig. 8).



**Figure 8.** Bayesian Inference tree using the concatenated alignment of COI and 16S. Analysis was run for 50 million of generations, sampling one tree every 1000 generations. Asterisks \* show sequences from Colombian specimens and donated by EVOLab, University of California, Berkeley; # belong to DNA aliquots sent from Jessica Garb. Branch numbers indicate posterior probability PP.



**Figure 9.** A: Haplotype network of the partial sequence of COI for *L. garbae* sp. nov., and related taxa. Two colors for *L. garbae* sp. nov.; green corresponds to the population from the Tatacoa desert, and blue to the population from Natagaima. B: Haplotype network of the partial sequence of 16SrRNA for *L. garbae* sp. nov., and related taxa. Two colors for *L. hesperus*; green corresponds to the population from Canada, and purple to the population from US. C: Haplotype network of the partial sequence of COI for *L. hurtadoi* sp. nov., and related taxa. D: Haplotype network of the partial sequence of COI for *L. hurtadoi* sp. nov., and related taxa.

The second group *L. hurtadoi* sp. nov. (MT2) was obtained from Mesa de los Santos (Barichara, Colombia), where it occurs roughly in the same area with *L. curacaviensis* and *L. geometricus*; it is sister to the two North American lineages currently recognized as *L. hesperus* Chamberlin and Ivie, 1935 (Fig. 8). *L. hurtadoi* sp. nov. is monophyletic (PP= 0.75) in the concatenated alignment (Fig. 8), and PP of 1 in both COI and 16SrRNA analysis (Figs. S1 and S2). The nearest species is the population of *L. hesperus* from Canada with a PP of 1. The population of *L. hesperus* from United States is outside of the clade (PP= 1), possibly showing a different species than the one recovered from Canada. This clade is related to *L. mactans* (Fabricius, 1775), *L. bishopi* (Kaston, 1938) and *L. variolus* (Walckenaer, 1837) with a PP of 1 but showing low internal resolution (PP= 0.36) in the concatenated analysis, while in the COI phylogenetic hypothesis the support is higher (Fig. S1). The position of *L. mactans* is not well resolved, having a different position in the inference using COI. Still the individuals were always recovered monophyletic in all analysis (Figs. 8 and S1).

In the *mactans* clade, *L. mactans* groups with *L. hesperus* clade with a pp of 0.44. The relation between *L. bishopi* and *L. variolus* is recovered in both trees with a pp of 0.88 in the concatenated alignment (Fig. 8) and of 0.92 in the COI alignment (Fig. S1). None of these species were included in the 16S tree. The next clade we can see in Fig. 8 is the *hesperus* clade, that includes *L. hesperus* and *L. hurtadoi*, sp. nov. showing three distinctive groups, *L. hesperus* USA, *L. hesperus* Canada, and *L. hurtadoi*, sp. nov., showing well resolved differences between the two populations of *L. hesperus*.

New populations of *L. garbae* sp. nov. were detected north of the Tatacoa desert in locations such as Natagaima, Castilla, Aipe and Saldaña, and one specimen was collected in La Dorada, Caldas. *L. hurtadoi* sp. nov. was only found in “La Mesa de los Santos”, in the same ecosystem with *L. curacaviensis* and *L. geometricus*, but the webs were not found near each other (Fig. 1). According to the analysis of COI, the genetic distance between *L. corallinus* and *L. diaguita* was of 0.1% with a Fst index of 0 and a Gst index of 0

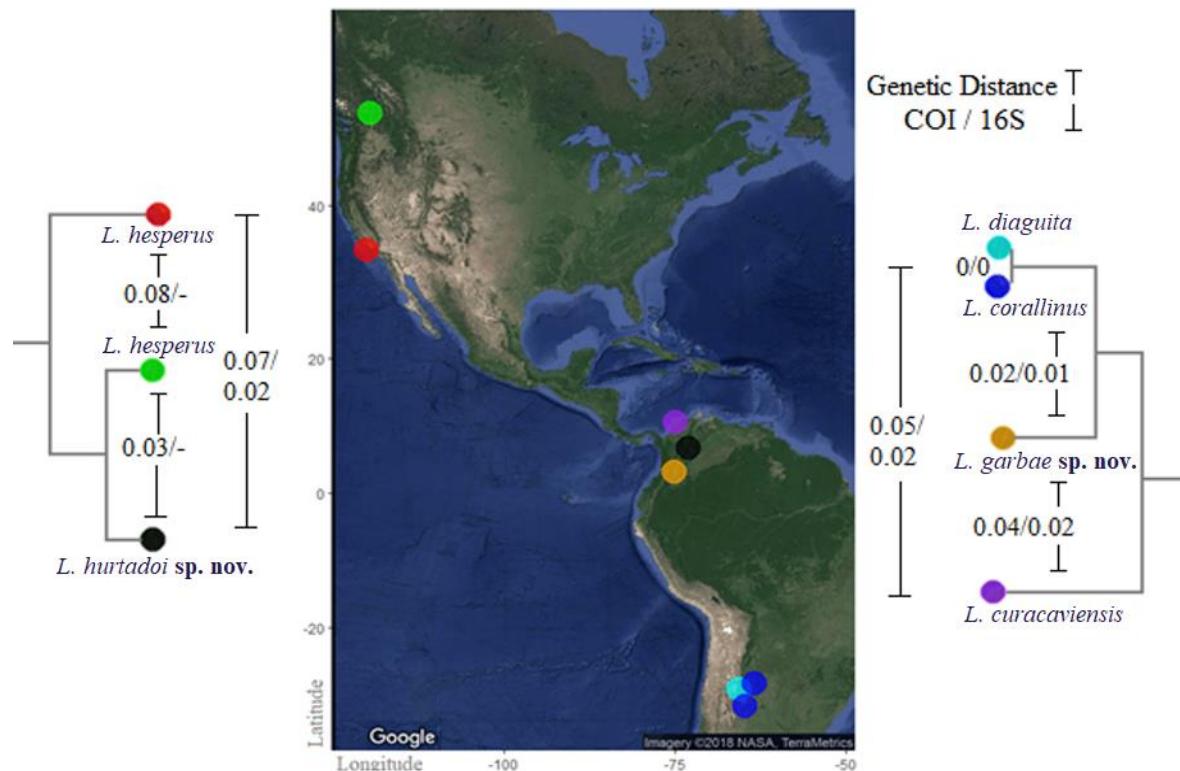
and a genetic flow Nm of 0.5. Between these two species and *L. curacaviensis* the distances were 5.1% and 5%, with Fst's of 0.97 and 0.99, and Gst of 0.31 and 0.63, respectively. The relation between *L. curacaviensis* and MT1 showed a genetic distance of 4.4% and and Fst of 0.96, Gst of 0.41. Between the Argentinian species, *L. corallinus*, *L. diaguita* and MT1 the minimum genetic distance was of 1.7% with an Fst value of 0.85 (Table S6). The haplotype network for COI (Fig. 9A) shows 16 fixed sites between *L. curacaviensis* and MT1, and 6 between the Argentinian species and MT1. Also, one haplotype between the two Argentinian species is shared. Alignments and fixed sites for COI Alignment can be seen in Table S2.

The haplotype network for the 16SrRNA analysis (Fig. 9C) show 7 fixed sites between *L. curacaviensis* and MT1, a genetic distance of 1.8%, and an Fst index of 1. Two diagnostic characters were obtained between MT1 and the two Argentinian species. The lower genetic distance recovered between the Argentinian species and *L. curacaviensis* was of 1.8% with an Fst index of 0.87. The lower genetic distance between MT1 and the Argentinian species was of 1.7% with an Fst of 0.87 (Table S6). Shared haplotypes are found in the two Argentinian species. To see the alignment and fixed sites see Table S3

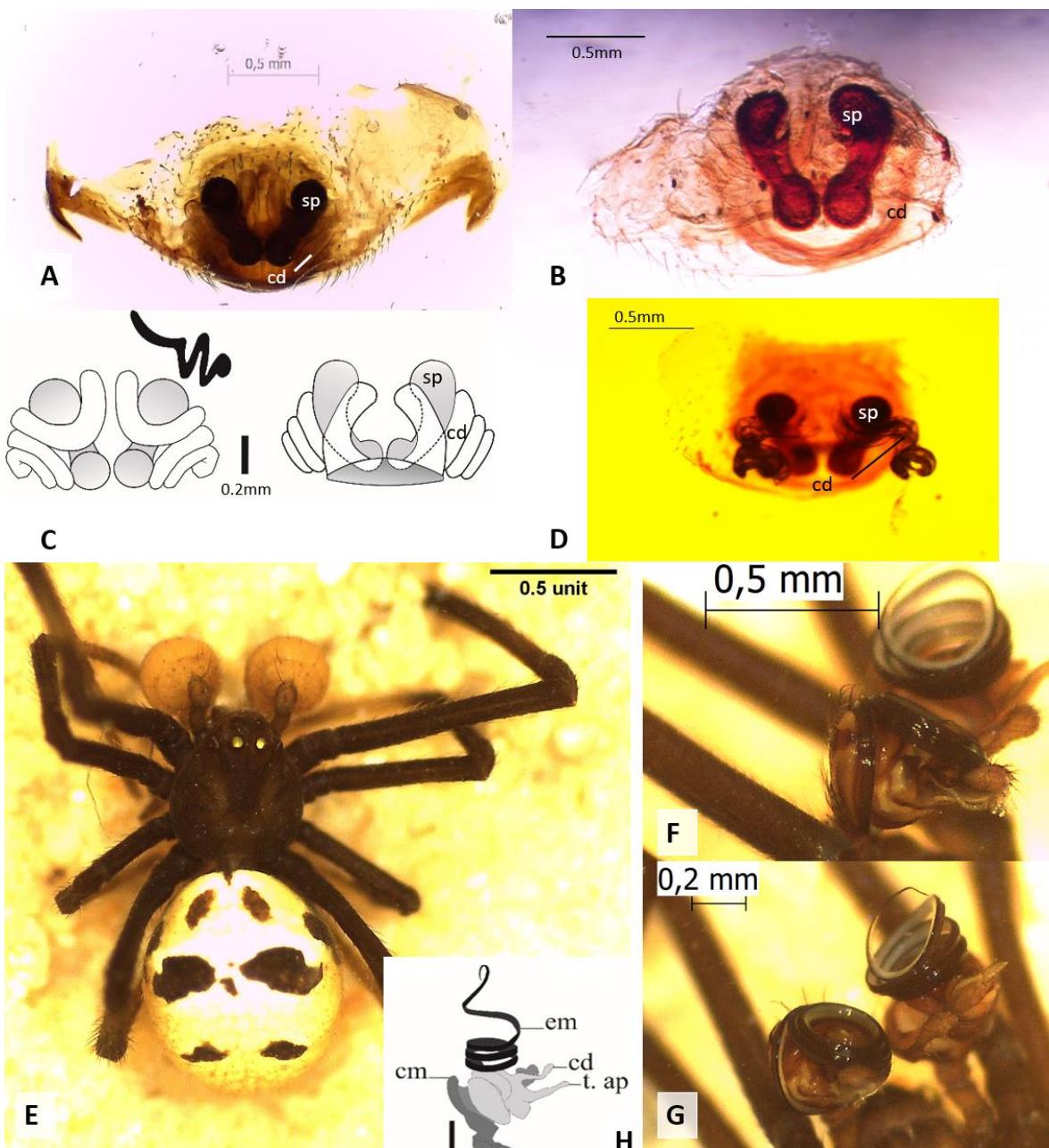
The *hesperus* clade, shows *L. hesperus* grouped with MT2 in three well defined groups for all analysis, and reciprocal monophyly was recovered for all trees (Figs. 8 and S2-S3). Using COI gene fragment, *L. hesperus* from Canada, separates from *L. hesperus* from United States with a genetic distance of 7.5%. The population of *L. hesperus* from Canada forms a monophyletic group with MT2 showing a genetic distance of 2.7% and a pp of 1 for all phylogenetic hypotheses. Also, there are 14 fixed sites between these two species (Fig. 9B and Table S4), and 30 between the two populations of *L. hesperus*. The K2P genetic distance recovered between the Canada population and the US population is of 7.5% with an Fst index of 0.94 (Table S6). 16SrRNA alignment between MT2 and related taxa showed a genetic distance of 1.6% and an Fst index of 0.96, haplotype network showed 7 fixed sites (Fig. 9D, Table S6).

Summarizing, the K2P genetic distances between our two unnamed lineages and their closest relatives were between 1.7–4.4% (Fig. 10 and Table S6). The genetic alignment for COI (Table S2) revealed between 6 and 16 fixed sites that separated the unnamed specimens of Tatacoa from the Argentinian species and *L. curacaviensis*, respectively.

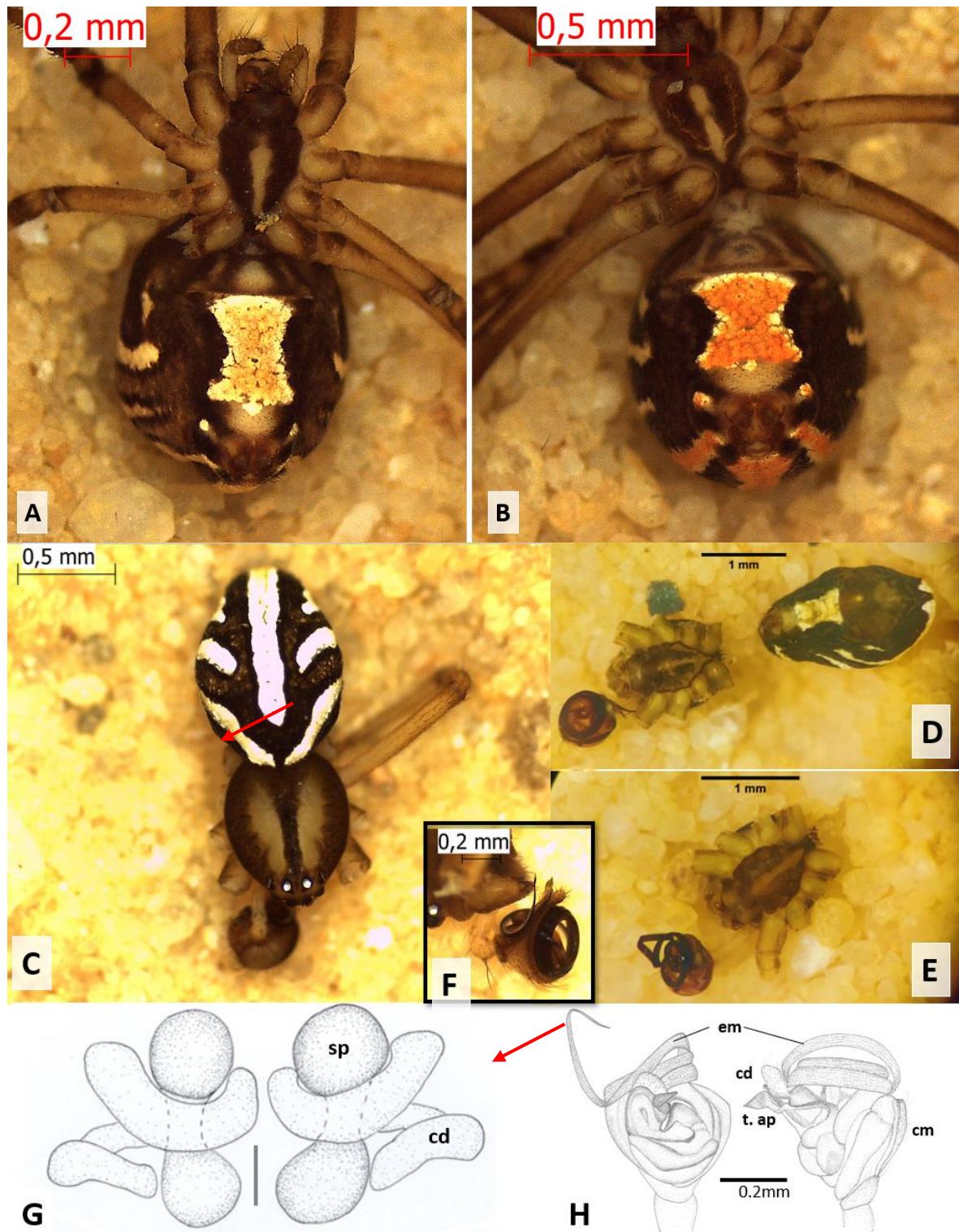
Based on the clear reciprocal monophyly between each of our two groups of unnamed spiders and other taxa, the long geographic distance, and the moderate to large genetic distance separating them and the high Fst values recovered, we used the additional morphological evidence to formally describe them as new taxa.



**Figure 10.** The type locality of the two new species of *Latrodectus* at Mesa de los Santos: *L. hurtadoi* sp. nov. (black dot) and Tatacoa: *L. garbae* sp. nov. (yellow dot), both in dry forests of Colombia. Distribution and K2P genetic distances to the phylogenetically closest species is also shown.



**Figure 11.** *L. garbae* sp. nov. Reproductive characters description A-B: Female epigynum showing 3 coils in the copulatory ducts (cd); C: Epigynum dorsal scheme with path scheme of spermatic ducts; D: Female epigynum showing 2 coils in the copulatory ducts (cd); E: Adult male; F-G: Right palp prolateral view. Embolus (em), conductor (cd), terminal apophysis (t. ap), cybium (cm); H: Pedipalp frontal scheme, scale bar=0.2mm.



**Figure 12.** *Latrodectus hurtadoi* sp. nov. A: Female spiderling ventral view; B: Female juvenile ventral view; C. Adult male, arrow points the first stripe; D: Adult male ventral view; E: Male adult extended pedipalp ventral view; F: Adult male, lateral view of the pedipalp. Reproductive characters description: G: Epigynum dorsal view with path scheme of spermatheca ducts, two coils present in the copulatory ducts (cd). Scale bar: 0.2 mm H. Right palp frontal and prolateral scheme. Embolus (em), conductor (cd), terminal apophysis (t. ap), cybium (cm).

## 4. DISCUSSION

The taxonomy of *Latrodectus* has been difficult given the highly conserved morphology among the species (Levi 1983), as well as the high intraspecific variation of coloration and genitalia: across-species comparisons have failed to find a character that works for the differentiation of species (Gerschman & Schiapelli 1965). Our description of male and female genitalia of *L. garbae*, sp. nov. and *L. hurtadoi*, sp. nov., revealed many similarities to other *Latrodectus*, even, the pedipalp and epigynal structures can be very similar to other species.

Molecular techniques support the description of new species, especially when species have multiple morphotypes, as in *Latrodectus* (Melic 2000). COI and 16SrRNA genes allow the discrimination and differentiation of black widow spiders (Croucher *et al.* 2004; Barrett & Hebert 2005; Padial *et al.* 2010). The COI marker has a good phylogenetic signal, but for intraspecific analysis does not have any resolution; moreover, the use of just one marker could bias phylogenetic analyses (Simon *et al.* 2006; Hajibabaei *et al.* 2007). In this study we used mitochondrial markers to improve the resolution of the phylogenetic inferences, but it is highly recommended the use of nuclear markers for further analyses. The trees presented here have a similar topology compared to Garb *et al.* (2004): two main clades are recovered in all trees, *geometricus* and *mactans*.

The *geometricus* clade groups all *L. geometricus*, regardless of the collecting site, and its sister species *L. rhodesiensis* Mackay, 1972. All *L. geometricus* from South America form a clade (PP= 0.85). The origin of this species is yet unknown, and its wide distribution and invasion history makes it difficult to determine (Marie & Vetter 2015). All the Colombian *L. geometricus* form a clade with a pp of 0.85. New population reports are made for the country in Meta, Cundinamarca, Vichada, Guajira, Bolívar, Tolima, and Santander. Also, a new report for *L. curacaviensis* in the Department of Santander. For specific localities see Table S1 and Fig. 1.

The genetic distance between *L. hesperus* from USA and Canada is of 8%, and it has been suggested in previous studies that they belong to different species (Barrett & Hebert 2005). Also, an Fst index of 0.95 shows high population structure and we recovered an Nm index of 0.01 that supports no gene flow between them, there are 32 fixed sites between the sequences of these two species in the COI gene fragment (Table S4). These results support the hypothesis that they belong to different species, but specimen revision is needed, and a deeper analysis is needed in order to determine the correct identity of these species.

*L. hurtadoi*, sp. nov. show a genetic distance of 7% with *L. hesperus* USA and of 3% with *L. hesperus* Canada based on COI. Also, the percentage of divergence for COI is of 5.79% and 2.51%. There are 30 and 13 fixed sites between these species and Fst indexes of 0.94 and 0.98, for COI and 16S respectively showing high population structure and no gene flow (Table S5 and S6). These analyses, with the reciprocal monophyly recovered for all trees, the morphological analysis, and the geographical location, supports the hypothesis of the new species status for *L. hurtadoi*, sp. nov.

The next well-defined clade is where the Australian *L. hasseltii* (Thorell, 1870) and *L. katipo* (Powell, 1870) group together. They are recovered with the concatenated alignment COI alone with a pp=1 for both, no 16S data was available for these species. The relation of this clade to other species is not well resolved; in the COI tree, *L. hasseltii* + *L. katipo* is sister to the remaining *Latrodectus* species (PP= 0.92), while in the concatenated analysis (*L. hasseltii* + *L. katipo*) is sister group to *L. tredecimguttatus* (Rossi, 1790) with a PP of 0.37. Also, the position of *L. menavodi* (Vinson, 1863) is not well resolved, as it changes also depending on the analysis and has low resolutions in both trees. *L. revivensis* (Shulov, 1948) is recovered as sister to *L. pallidus* (Cambridge, 1872) in both analysis with a pp=1 for the two trees, but the external relations to *L. menavodi* and *L. tredecimguttatus* are not well resolved. The phylogenetic relationships among non-Colombian *Latrodectus* species should be assessed with an augmented taxon and character sampling.

The South American clade shows that *L. mirabilis*, *L. variegatus* and *L. thoracicus* group together with a pp=1 for all trees. This clade should be studied deeper, results show that *L. thoracicus* and *L. variegatus* may belong to the same species; type specimens should be checked, and deeper genetic analyses made. The position of *L. antheratus* (Badcock, 1932) changes between concatenated, COI and 16SrRNA inference trees. In the concatenated hypothesis and COI analyses it forms a monophyletic clade with the Colombian species (pp=0.94, pp=0.87 respectively), but in the 16SrRNA inference tree the topology supports its position next to *L. mirabilis* and *L. variegatus* with a pp=0.64. The *curacaviensis* clade groups *L. curacaviensis*, *L. corallinus*, *L. diaguita* and *L. garbae* sp. nov. The internal topology of this group was recovered as reciprocal monophyly in all trees with the lowest pp of 0.99. *L. curacaviensis* appears as sister of the other species (pp=1 in all topologies). *L. corallinus* and *L. diaguita* appear together with internal topologies not defined. The genetic distance between these two Argentinian species is 0% and they show a value of Fst of 0 and a 0% in genetic divergence; also, there are no fixed sites between these two species. The specimens collected in Garb *et al.* (2004) showed a difference in the egg sac morphology, as described in Abalos (1980), where *L. corallinus* shows a “spiked” egg case, while *L. diaguita* has a smooth egg case; there are no genetic differences, suggesting that these two species are synonyms, more studies are needed, and specimens should be checked. *L. garbae* sp. nov. shows a genetic distance of minimum 2% and a minimum Fst

value of 0.86 with very low gene flow and at least 5 fixed sites for COI and 2 for 16SrRNA. Reciprocal monophyly was recovered for all trees and geographical location changes.

*L. curacaviensis* has been poorly studied due to the loss of the type specimen. In Colombia the species is established in the north Andean zone, specifically in dry forests, far away from human constructions. The habitat loss and competence with other species, including other *Latrodectus* could be playing an important role in the segregation the populations have been through. This research gives insight about the actual populations of *L. curacaviensis*, not reported in the literature before.

The morphological analyses show poor differences between the candidate species and the species already described, that include general morphology of reproductive structures, color patterns and morphological measurements, although there is no morphological support, the genetic analyses support the hypothesis of the new species status for *L. garbae* sp. nov. and *L. hurtadoi* sp. nov. Altogether, our molecular data and phylogenetic hypothesis contribute to the knowledge and increase the species sampling for COI and 16S markers in the genus.

## TAXONOMY

Order Araneae Clerck, 1757

Family Theridiidae Sundevall, 1833

*Latrodectus* Walckenaer, 1805

*Latrodectus garbae* new species, Rueda and Realpe

**Type Material: Holotype:**

f# (ANDES-IN2987) COLOMBIA, Huila Department Villa Vieja Municipality, (3°13'0.23"N, -75°8'0.72"W; 502 m), Tatacoa desert, 11-Feb-2008, F. Borrero, under a rock, by hand. Paratypes: 1 f# (ICN-Ar-8124) same locality as holotype, 24-June-2015, A. Rueda, under a rock, by hand; 1 m# (ICN-Ar-8125) same locality as holotype, 24-Jun-2015, A. Rueda, under a rock, by hand, not dissected.

## Etymology:

The species epithet, a noun in apposition, honors Jessica Garb, scientist who built the first molecular phylogeny of the genus and has greatly contributed to the knowledge of black widow systematics and evolution.

## Diagnosis:

No distinct or unique feature of the male palp and female epigynum were found that reliably diagnose *L. garbae* from other *Latrodectus* species. *L. garbae* can be diagnosed from other related *Latrodectus* species, based on the following synapomorphic mtDNA nucleotide substitutions at the DNA barcode alignment positions in each species (Table S2):

COI ◊ Curacaviensis group: *L. garbae*: G (6), A (39), G (48), A (108), A (117), A (147), C (203), T (237), A (264), A (273), A (321), T (328), A (351), C (357), C (375), C (390). *L. curacaviensis*: A (6), G (39), A (48), G (108), G (147), T (203), C (237), T (264), G (273), G (321), C (328), G (351), T (357), T (375), T (390). *L. diaguita* and *L. corallinus* share the DNA substitutions: C (126), G (116), G (261), C (334), T (390), T (402).

16SrRNA ◊ Curacaviensis group: *L. garbae*: A (260). *L. curacaviensis*: T (81), T (115), C (216), G (223), C (234), A (248). *L. diaguita* and *L. corallinus* share the DNA substitutions: A (190).

## Description:

Female spermatheca have three coils in *L. garbae*, the spermatheca are dumbbell shaped with the ends of different sizes, the first one is greater than the second (Fig. 11). Male embolus has three coils; conductor distal part with a pronounced curvature to the prolateral direction (Fig. 11). Black ventral abdomen with the red hourglass characteristic of the genus (Figs. 4D and 4G). Shape of the hourglass not as marked as in other *Latrodectus* species, but more rounded. Red dorsal abdomen with six easily recognizable spots forming a symmetrical arrangement in the antero-posterior axis (Fig. 4C). The spots located near the pedicel are the largest ones and can get fused with the black background of the pedicel portion during ontogeny, (Fig. 4B), and the medial sports can get fused with the lateral black section of the body (Fig. 4E) forming an arrow-like structure, which points to the anterior portion of the abdomen (Fig. 4F); the smallest spots are in the posterior portion of the abdomen body and can be variable in size, from small black points (Fig. 4B) to dark oval very noticeable spots (Fig. 4C). Males show similar color patterns in the abdomen, but the spots do not fuse, they show a variable number of sports maintaining the symmetrical arrangement of the six spots in the antero-posterior axis. The color and the pattern in the abdomen are retained until maturity (Fig. 11). **Female:** Small size spider. Bright brown carapace with dark brown color in thoracic furrow and radial furrow, equally long as wide (cephalothoracic coefficient  $T = 1.04 \pm 0.08$ ) with oval shape. **Measurements (mm):** Female (holotype): Total body length: 6.65. Carapace length: 2.56; width: 2.33. Cephalothoracic

coefficient: 1.10. Femur I length: 4.85. Patella I length: 1.26. Tibia I length: 3.81. Sternum length: 1.45; width: 1.21. Male (paratype): Total length: 2.74. Carapace length: 1.01; width: 1.06. Carapace coefficient: 0.95. Tibia I length: 2.24. Patella I length: 0.57. Sternum dark brown to black. Abdomen sub globular with distinctive color pattern, totally covered with setae. Legs dark brown to black with joints of the same color. Epigynum heavily sclerotized, epigynal plate with sub oval shape, wider than long, covered with thin setae, oval opening of the atrium with sclerotized margins. Spermatheca dumbbell shaped forming a V structure, close together in the base but not in contact (Fig. 11C). Copulatory ducts with two to three coils, with the last coil is shorter than the others, when the duct is expanded, the distal portion is observed to have a circular flattened form, membranous like, and dorsally projected. **Male:** Male size much smaller than females. **Measurements (mm):** Male: Tibia I length: 2.8, Cephalothorax length: 1.0, Cephalothorax width: 1.1. Dark brown carapace equally long as wide (cephalothoracic coefficient mean T:  $0.96 \pm 0.01$ ). Sub globular abdomen with color pattern similar to that of females. Dark brown legs in proportion to the body much longer than females. Pedipalp similar to *L. curacaviensis*, embolus with three coils, same as copulatory ducts in females.

#### Egg Sac:

In 10 egg sacs we inspected, the shape was spherical, with wooly appearance and white coloration, and the surface did not have ornamentation. Average diameter of  $8.02 \pm 0.16$  mm.

#### Variation:

One out of 10 examined female epigynums presented just two coils in the spermatic ducts.

#### Distribution:

Inter-Andean Magdalena Valley of southwestern Colombia, in Huila, Tolima and Caldas Departments. Found in hot and dry places, away from human structures and near to the ground in small vegetation or rocks (Figs. 4E-I). *Latrodectus garbae*, sp. nov. was first found in 2009 living in sympatry with *L. geometricus* in Tatacoa. After 2016, no specimens were found again, but new populations were detected north to the type locality, in Natagaima, Castilla, Aipe and Saldaña; and one specimen was collected in La Dorada, Caldas.

#### Natural history:

Spiders were found near the floor, with webs reaching a mx height of 60 cm. No nest was built when the webs where found on sand or rocks, but when they were found under leaver, organic material was used to create a “balloon” under which spiders would hide.

#### Additional specimens studied:

Material examined includes 20 f# and 10 m# from the same locality as holotype, collected by Alexandra Rueda and Emilio Realpe, (ANDES-IN 2988:2995-2998:3005-5202:5203-5341-5351). All measurements in mm. **Females (N=20):** Carapace length:  $2.43 \pm 0.31$  (mean  $\pm$  sd) (range 1.85 – 3.17); width:  $2.33 \pm 0.20$  (range 2.03 – 2.73). Cephalothoracic coefficient:  $1.04 \pm 0.08$  (range 0.81 – 1.16). Femur I length:  $4.55 \pm 0.46$  (range 3.75 – 5.39). Patella I length:  $1.16 \pm 0.15$  (range 0.91 – 1.47). Tibia I length:  $3.79 \pm 0.40$  (range 3.30 – 4.84). Sternum length:  $1.51 \pm 0.15$  (range 1.29 – 1.92); width:  $1.28 \pm 0.14$  (range 1.10 – 1.54) (Table 1 and 2) . **Males (N=10):** Total length:  $2.74 \pm 0.19$  (range 2.32 – 3.22). Carapace length:  $1.02 \pm 0.12$  (range 0.92 – 1.27); width:  $1.07 \pm 0.13$  (range 0.94 – 1.32). Carapace coefficient:  $0.95 \pm 0.06$  (range 0.73 – 1.04). Tibia I length:  $2.25 \pm 0.3$  (range 1.0 – 2.87). Patella I length:  $0.58 \pm 0.11$  (range 0.46 – 0.67). Patella- Tibia index:  $2.77 \pm 0.90$  (range 1.55 – 3.31) (Table 3).

#### Delimitation with other *Latrodectus* species:

No statistical analysis was performed due to the small number of measurements available for *L. corallinus* and *L. diaguita*. Results (Figs. 6 and 7, Table S7, S8 and S9) show that the smaller legs belong to *L. curacaviensis* with average values of 4.2 for femur, 4.4 for Patella-Tibia and 5.7 for Metatarsus-Tarsus; followed by *L. garbae* sp. nov. with values of 4.6 for femur, 4.9 for Patella-Tibia and 6.1 for Metatarsus-Tarsus. *L. corallinus* is next in the list with values of 5.1 for femur, 5.1 for Patella-Tibia and 7.0 for Metatarsus-Tarsus, these values were obtained from Abalos (1980) and they correspond to the mean of two individuals. The measurements recorded for *L. diaguita* show a big specimen that could correspond to an outlier, since we could only access the information for one specimen, there is no way of getting a confirmation for the mean size of this species. The cephalothorax average measurements show similar results, *L. curacaviensis* is the smallest species with a mean of 2.3 for cephalothorax length and 2.1 for cephalothorax width. The next species in size is *L. garbae* sp. nov. with a mean of 2.4 for cephalothorax length and 2.3 for cephalothorax width

(Table 1 and 2; Fig. S10). These two species are followed by *L. corallinus* with a mean of 3.0 for cephalothorax length and 2.7 for cephalothorax width. *L. diaguita* only had reports of measurement for cephalothorax width with a size of 3.2 for a single specimen.

#### *Latrodectus hurtadoi* Rueda and Realpe, sp. nov.

##### Type Material: Holotype:

f# (ANDES-IN5234) COLOMBIA, Santander Department, Mesa de los Santos Municipality, (6°47'54.6714"N, 73°9'46.6554"W; 1400 m), 27-Sept-2014, A. Rueda, E. Realpe, under a rock, by hand. Paratypes: Eight f# (ANDES-IN5235, 5636, 5238, 5241, 5243, 5247:5249) same information as holotype. 1 m# (ANDES-IN5799), 15-Mar-2015, A. Rueda and E. Realpe, reared in laboratory.

##### Etymology:

The name of the species it's a dedication to Andrés Hurtado García, Colombian journalist, ecologist, alpinist, and nature photographer. In honor to his great labor in Colombian education and environment protection activities, and because of his passion for life and spiders.

##### Diagnosis:

No distinct or unique feature of the male palp and female epigynum were found that reliably diagnose *L. hurtadoi* from other *Latrodectus* species. *L. hurtadoi* can be diagnosed from other related *Latrodectus* species, based on the following synapomorphic mtDNA nucleotide substitutions at the DNA barcode alignment positions in each species (Table S2):

COI ◊ Hesperus group: *L. hurtadoi*: A (54), T (63), A (153), C (276), G (382), T (408), A (432), C (493). *L. hesperus* from Canada: G (39), G (123), C (297), T (358), G (408), C (499). *L. hesperus* from US: A (66), A (105), A (225), A (228), T (252), G (255), C (259), A (273), A (306), A (317), A (360), A (408), G (417), G (423), C (427), T (431), A (435), G (441), A (477), A (492), A (501), T (504), A (507), A (513).

16SrRNA ◊ Hesperus group: *L. hurtadoi*: T (5), A (61), T (112), T (196), C (228), G (370). *L. hesperus* from US: C (5), G (61), A (112), C (196), T (228), A (370).

##### Description:

Genitalia is similar to other *Latrodectus* species. In all females dissected, the male embolus was recovered inside the epigynum.

**Measurements (mm):** f# (holotype): Carapace length: 2.88 mm; width: 2.41. Cephalothoracic coefficient: 1.19. Femur I length: 4.70. Patella I length: 1.59. Tibia I length: 4.65. Sternum length: 1.74; width: 1.38. m# (paratype): Carapace length: 1.6; width: 1.3. Carapace coefficient: 1.2. Tibia I length: 2.5. Patella I length: 0.7. The color pattern in females is very similar to *L. hesperus*, even in the ontogenetic change. Spiderlings show a light brownish coloration, similar to *L. geometricus*, but the color pattern in the abdomen show lateral orange, red or white stripes surrounded by a darker border. The first stripe, near the pedicel goes around the abdomen and ends up in the ventral portion (Figs. 5A and 12A). Second and third stripe follow the lateral segment of the body, and the third and bigger goes from the spinnerets to the middle of the dorsal portion in the abdomen, following the middle longitudinal axis of the body. Carapace light brown with a darker border and a longitudinal darker line going through the carapace furrow (Fig. 5). Legs light brown with darker portions in joints and in the middle of each segment (Fig. 5B). After a couple of molts, the abdominal background color turns darker and the lateral stripes start to fade. The longitudinal stripe turns reddish (Figs. 5B and 12B). When maturity is reached the longitudinal red stripe is retained; also, the transversal stripe near the pedicel, can or cannot be present in an adult female (Figs. 5C, 5D and 5E). The legs and cephalothorax turn dark brown to black. The shape of the hourglass is marked and noticeable (Fig. 5D). Male embolus has three coils, showing a loop in the end of the third coil (Fig. 12F and 12H). The conductor and the terminal apophysis are close together, and both show pronounced curvature to the prolateral direction (Fig. 12F and 12H). Males keep the spiderling coloration until maturity (Fig. 12C). **Female (N=20):** Big size spiders. Bright black to dark brown carapace, with dark black color in the thoracic furrow and radial furrow, longer than wider (cephalothoracic coefficient T = 1.12 ± 0.09) with oval form. Black sternum longer than wide. Sub globular abdomen with distinctive color pattern, totally covered with setae. Dark legs with joints of the same color or with darker joints and darker marks in each segment. When they are reaching sexual maturity the color pattern is lost, leaving only the red line across the abdomen and the transversal line near the pedicel (Fig. 5C). The hourglass is big and defined on a black background (Fig. 5D). The epigynum (N=10) is sclerotized, epigynal plate in sub oval form with the borders well defined, wider than long covered with thin setae, oval opening of the atrium with sclerotized margins. Dumbbell spermathecae in V structure, close together in the base but not in contact (Fig. 12G). Copulatory ducts have two to three coils, when present the third coil is located in the back of the spermathecae and is shorter than the other two. All of the dissected epigyna had the male coils inserted (males' loose structure during copula). **Male:** Just one male reared in captivity (Fig. 12C). **Measurements (mm):** Size much smaller than females (3.56 mm). Light brown carapace longer than wider (cephalothoracic

coefficient T: 1.26). Subglobular abdomen with coloration similar to spiderlings. Light brown legs in proportion to the body much longer than females. Males keep the light brown coloration all their life.

#### Egg sac:

(N=6) Spherical white egg sac with wooly organized appearance, the surface does not have ornamentation. Average diameter of  $7.56 \pm 0.98$ mm.

#### Distribution:

Northern Colombia, Department of Santander, Mesa de los Santos, located in hot, dry places away from human structures and near to the ground in small rocks; the habitat lacked tree or shrub vegetation and was mostly sandy. All the localities and sampling sites for each species are found in Table S1.

#### Natural history:

*L. hurtadoi, sp. nov.* was only found in "La Mesa de los Santos", in the same ecosystem with *L. curacaviensis* and *L. geometricus*, but the webs were not found near each other. The spiders were always away from human roads or constructions, in a field full of little stones on the ground. The webs were hidden under rocks, and spiders were not easily visible.

#### Additional specimens studied:

Material examined includes 12 f# from the same locality as holotype, collected by Alexandra Rueda and Emilio Realpe; and 2 m# from the same locality as holotype, collected by Emilio Realpe (ANDES-IN5762, 5764, 5767, 5768, 5771, 5774, 5779, 5780, 5787:5789, 5791). **Females (N=20):** Carapace length:  $2.90 \pm 0.43$  (range 1.63 – 3.64); width:  $2.61 \pm 0.37$  (range 1.37 – 3.02). Carapace coefficient:  $1.12 \pm 0.09$  (range 0.91 – 1.26). Femur I length:  $5.00 \pm 0.48$  (range 3.80 – 5.88). Patella I length:  $1.50 \pm 0.17$  (range 0.96 – 1.77). Tibia I length:  $4.17 \pm 0.41$  (range 3.38 – 4.97). Sternum length:  $1.94 \pm 0.32$  (range 1.40 – 2.60); width:  $1.54 \pm 0.31$  (range 1.23 – 2.60) (Figure 6 and Table S7). **Male (N=3):** Total length: 3.56. Carapace length: 1.29; width: 0.78. Carapace coefficient: 1.21. Tibia I length: 2.53. Patella I length: 0.71. Patella-Tibia index: 2.08 (Figure 7 and Table S10).

#### Delimitation with other *Latrodectus* species:

No statistical analysis was performed due to the small number of replicas for USA and Canada specimens. Leg I measurements were made for the *hesperus* clade including the species *L. hurtadoi* sp. nov. (N=20), and *L. hesperus* (N=10). Results (Fig. 6 and Fig. 7, Table S7) show that the smaller legs belong to *L. hurtadoi* sp. nov. with values of 2.79 for femur, 0.67 for Patella, 2.12 for Tibia, 3.17 for Metatarsus and 1.08 for Tarsus; *L. hesperus* show measurements of 3.36 for femur, 0.79 for Patella, 2.92 for Tibia, 3.66 for Metatarsus and 1.51 for Tarsus. The caparace average measurements (Table 1 and 4; Fig. S12) show similar results, *L. hurtadoi* sp. nov. is the smallest species with a mean of 1.43 for cephalothorax length, 1.18 for cephalothorax width, 0.83 for sternum length and 0.55 for sternum width; *L. hesperus* show measurements of 1.45 for cephalothorax length, 1.27 for cephalothorax width, 1.01 for sternum length and 0.63 for sternum width. Male cymbium was also measured to find differences (Fig. 7, Table S10), *L. hurtadoi* sp. nov. shows a length of 0.74 and a width of 0.72, while *L. hesperus* shows a length of 0.67 and a width of 0.79. The cymbium of *L. hurtadoi* sp. nov. is longer, while the cymbium of *L. hesperus* is wider (Table S10).

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#### Conflicts of interest:

The authors declare no conflict of interest.

**Ethical approval**

The Animal ethical guidelines are followed in the study for species observation & identification.

**Data and materials availability**

All data associated with this study are present in the paper. For supplementary materials, visit URL:

[https://www.discoveryjournals.org/Species/current\\_issue/2021/v22/n70/A9S.pdf](https://www.discoveryjournals.org/Species/current_issue/2021/v22/n70/A9S.pdf)

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